Effects of *Meloidogyne hapla* and *M. incognita* on *Phytophthora* Root Rot of Alfalfa

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ABSTRACT

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Sequential inoculation with either *Meloidogyne hapla* or *M. incognita* and *Phytophthora megasperma* f. sp. *medicaginis* intensified Phytophthora root rot severity in alfalfa cultivars resistant and susceptible to *Phytophthora*. *M. hapla* was more effective than *M. incognita* in exacerbating root rot symptoms, but both nematode species suppressed top and root growth, increased chlorosis, and reduced plant vigor. Inoculation with *Phytophthora* reduced the root mass available for nematode reproduction.

Additional key words: nematode-fungus interactions

Phytophthora megasperma Drechs. f. sp. *medicaginis* (as proposed by Kuan and Erwin [15]) causes a serious root rot of alfalfa (*Medicago sativa* L.) and limits yield and persistence on poorly drained soils. The disease was first described by Erwin (7,8), and has since been reported in alfalfa-growing regions in the U.S.A. (6,9,14,17,25), Australia (13,23), Canada (4,5), and Japan (18). The fungus is likely to cause problems wherever alfalfa is grown commercially.

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Four species of root knot nematodes are considered pathogens of alfalfa, northern root knot (*Meloidogyne hapla* Chitwood), southern root knot (*M. incognita* (Kofoid & White) Chitwood), Javanese root knot (*M. javanica* (Treub) Chitwood), and peanut root knot (*M. arenaria* (Neal) Chitwood) (11). The growth of alfalfa is retarded more severely by *M. hapla* than by *M. incognita* (3,19), and *M. javanica* and *M. arenaria* are not considered to be economically important on this crop (11).

The detrimental influence of combined inoculations with root-infecting fungi and plant parasitic nematodes is well documented (21). An important disease complex between *P. parasitica* var. *nicotianae* and *Meloidogyne* spp. has been extensively investigated on tobacco. These nematodes encourage the development of the black-shank disease on susceptible tobacco and also predispose resistant cultivars to black-shank disease (20–22). Other possible disease complexes involving *Phytophthora* spp. and root knot nematodes on crops such as soybean (26) have received only limited study. Disease complexes of alfalfa involved root knot nematodes and soilborne pathogens that cause Fusarium (16) and bacterial wilts (12).

The purpose of this investigation was to determine whether *M. hapla* and *M. incognita* form disease complexes with *P. megasperma* f. sp. *medicaginis* that alter the reaction of resistant and susceptible alfalfa cultivars to Phytophthora root rot.

MATERIALS AND METHODS

We used alfalfa cultivars Saranac, Agate, and Apollo. In the 1978 evaluation in the Phytophthora root rot nursery at St. Paul, MN, their resistance to root rot was Saranac, 2%; Agate, 43%; and Apollo, 40% (1). Saranac is considered susceptible to Phytophthora root rot, and Agate and Apollo are considered resistant. Before planting, the seed coats of certified seed were scarified to permit rapid germination. Seeds were inoculated with an appropriate strain of *Rhizobium meliloti* and planted in fumigated sandy loam and sand (1:1, v/v). The seedlings were watered and

fertilized as needed to maintain vigorous growth at 16–21 C and were thinned to four per 10-cm diameter pot after 14 days.

Inocula of *M. hapla* and *M. incognita* were increased on well established Floradel tomato seedlings growing in a 1:1 mixture of loamy sand and soil in 15-cm diameter pots. Nematode eggs used to inoculate the alfalfa seedlings were extracted from the tomato roots after 8–12 wk of growth using the sodium-hypochlorite method (2).

The fungal inoculum was increased by growing each of four isolates of *P. megasperma* f. sp. *medicaginis* in 50-ml filtered V-8 juice broth for 14 days at 24–26 C. Three isolates were from diseased alfalfa growing in Madison, Rowan, and Wake counties in North Carolina and the fourth, from Minnesota, was provided by F. I. Frosheiser. In previous tests (25; R. E. Welty, *unpublished*, all isolates were highly virulent on susceptible alfalfa plants. After incubation, the medium was poured from the flask and the mycelium mat was washed once with distilled water. Single mycelial mats of each of four isolates were combined in 400 ml of distilled water and fragmented in a food blender. Six 50-ml samples of the inoculum were filtered through tared filter paper, dried overnight at 50 C, and weighed; the average dry weight of mycelium in 50 ml was computed for the six samples (41 ± 2 mg per sample).

After 28 days of incubation at 16–21 C, pots were moved to a greenhouse at 24–32 C. Each pot containing four plants was inoculated with 20,000 eggs of either *M. hapla* or *M. incognita*. Eggs in an aqueous suspension (30 ml) were poured onto the soil surface, then watered into the soil with an additional 50 ml of water. A 2- to 5-cm layer of fine sand was added over the soil surface.

Pots receiving the fungal treatments were infested with the washed mycelium of *P. megasperma* f. sp. *medicaginis*, 42 days after infection with nematodes. A 50-ml suspension was poured onto the soil surface and watered into the soil with another 50 ml of water.

After fungal inoculations, saucers under all pots were filled with water on each of 6 days to keep the soil saturated. This was followed by alternating 2–4 day periods of flooding and draining to provide conditions that favor development of Phytophthora root rot (24).

Plant growth responses, disease ratings, and nematode assays were recorded 28 days after the inoculations with fungi. The tops were rated for general appearance and chlorosis, and the length of the longest stem was measured. Then, the tops were removed by cultivar for each treatment and weighed separately. The soil was washed

Table 1. Effects of *Meloidogyne hapla* or *M. incognita* alone and combined with *Phytophthora megasperma* on growth of alfalfa,[a] root rot development, root galling, and nematode reproduction

Treatment / Cultivar[a]	Foliage				Root					
	Height (cm)	Top (g)	Chlorosis[b]	General appearance[c]	Root (g)	Tap root necrosis[d]	Secondary root necrosis[e]	Root knot galling[e]	No./10-cm pot Larvae	No./10-cm pot Eggs[f]
Control										
Apollo	73	27.2	4.7	8.7	26.2	1.0	1.7	1.7	0	0
Agate	78	25.3	4.0	8.2	28.5	1.0	2.8	1.6	0	0
Saranac	80	28.9	4.5	9.2	29.5	1.0	4.7	3.3	0	0
P. megasperma f. sp. *medicaginis*										
Apollo	70	23.2	3.5	8.0	22.3	3.0	39.8	0.0	0	0
Agate	66	18.2	3.7	7.8	20.2	2.3	34.0	3.3	0	0
Saranac	71	13.3	2.3	7.2	16.3	4.2	67.3	1.7	0	0
M. hapla										
Apollo	69	24.3	3.3	5.8	28.3	2.0	27.5	41.7	62	2,860
Agate	60	21.3	3.8	5.5	29.2	1.7	21.8	43.3	57	19,996
Saranac	71	25.0	3.3	6.0	28.5	2.0	21.7	40.0	67	1,302
M. incognita										
Apollo	67	19.8	3.7	7.3	18.5	2.0	27.7	13.3	173	490
Agate	65	21.5	4.2	7.8	21.3	1.2	13.0	11.7	63	1,191**
Saranac	78	25.0	4.2	8.3	28.2	1.2	13.7	11.7	122	2,186
M. hapla plus *P. megasperma* f. sp. *medicaginis*										
Apollo	59	9.5	1.8	4.8	14.7	4.2	77.0	28.3	17	199
Agate	59	14.5	3.0	6.4	28.0	4.0	65.9	35.0	40	1,107**
Saranac	65	11.3	2.0	6.2	14.5	4.5	79.2	28.3	92	330
M. incognita plus *P. megasperma* f. sp. *medicaginis*										
Apollo	74	18.2	2.5	7.2	22.3	3.7	58.3	11.7	192	27*
Agate	67	21.5	3.7	6.7	22.7	3.2	48.8	15.0	137	2,009*
Saranac	70	14.5	2.8	7.2	17.5	3.7	72.2	13.3	158	44*
LSD 0.05										
Apollo	10.2	8.1	0.9	1.3	10.9	1.2	21.4	6.2	162	
Agate	11.5	7.9	1.3	2.1	11.7	0.9	18.5	7.9	63	
Saranac	15.2	8.4	1.2	2.2	11.2	0.9	19.6	7.0	151	

[a] Each value is the average of six replications.
[b] 1 = complete, 5 = none.
[c] 1 = least vigorous, 10 = most vigorous.
[d] 1 = none, 5 = complete.
[e] 0 = none, 100 = maximum.
[f] Statistical analysis done with log-transformed data; asterisks (*,**) indicate significant difference from *M. hapla* treatments at $P = 0.05$ and 0.01, respectively.

from the roots, and roots were weighed per replicate. The root systems of the four plants per pot were rated 0–100 with a subjective score for galls and necrosis. Each taproot was rated for lesions and necrosis (10). Nematode eggs were extracted from the roots by the sodium-hypochlorite method (2). Second-stage larvae and males were extracted by a combination of elutriation and centrifugation (2).

The experiment was arranged in a split-plot design with six treatments (whole plot) and three cultivars (split-plot) with six replications, and the data were statistically analyzed for variation. The experiment was done three times with similar results, and the data from the last experiment are presented.

RESULTS AND DISCUSSION

Plants of all three cultivars inoculated with *P. megasperma* f. sp. *medicaginis* had more root rot and taproot necrosis than the controls (Table 1). This fungus also exacerbated foliar chlorosis of Apollo and Saranac. Root and top weights of Saranac were less for inoculated than for control plants. Agate was stunted, but effects of the fungus were less severe in Apollo and Agate than in Saranac. The appearance of the plants in all three fungus-inoculated cultivars was generally poorer than that of the controls, but the differences were not significant. These overall results generally confirmed that Agate and Apollo are more resistant than Saranac to Phytophthora root rot.

Both nematode species caused root galling and reproduced on all three cultivars. Based on the root galling, egg counts, and general appearance of the host, *M. hapla* caused more damage than *M. incognita*. This observation confirms early reports (3,19). Both nematodes stunted Agate and exacerbated chlorosis of Apollo. Neither nematode significantly suppressed shoot or root growth of Apollo. Nevertheless, both *M. hapla* and *M. incognita* predisposed Apollo to root rot by other unknown soil microorganisms. Only *M. hapla* increased root rot in Agate in control pots. These findings support the earlier observation that nematodes may predispose roots to rotting by soilborne fungi that otherwise are considered saprophytes (21).

When inoculated with *M. hapla* and then with *P. megasperma* f. sp. *medicaginis*, Apollo and Agate plants were stunted, top growth of Apollo and Saranac was reduced, and root rot and lesions increased in the taproots of all three cultivars. Root knot galls were found on the roots of all cultivars, chlorosis increased, and the general appearance of Apollo and Saranac was poorer than that of the controls. The combined inoculations with these two pathogens were detrimental to the host in all criteria of disease severity and plant growth or vigor.

As observed with *Meloidogyne* spp. in other disease complexes (21), the final nematode populations often were smaller after inoculation with the fungus and nematodes than after inoculation with nematodes only. This influence was particularly striking with egg counts for *M. hapla* on Agate.

When *P. megasperma* inoculation followed inoculation by *M. incognita*, the effects were generally similar to those with *M. hapla* but were less marked; stunting and chlorosis was less severe, general appearance was better, roots were generally larger, root necrosis was less marked, and fewer root galls were observed. Although *M. hapla* is more effective than *M. incognita*, both nematodes increased the damage caused by *P. megasperma* f. sp. *medicaginis*.

This study demonstrated that root knot nematodes predisposed both *Phytophthora*-resistant and -susceptible cultivars to root rot caused by *P. megasperma* f. sp. *medicaginis*. In areas where native root knot nematodes, especially *M. hapla*, are prevalent, cultivars that are resistant to Phytophthora root rot but susceptible to root knot might neither yield as well nor persist as long as cultivars with combined resistance to both nematodes and fungus.

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LITERATURE CITED

1. ANONYMOUS. 1978. Varietal trials of farm crops. Univ. Minn. Agric. Exp. Stn. Misc. Rep. 24. 32 pp.
2. BARKER, K. R., chairman. 1978. Determining nematode population responses to control agents. Pages 114-125 in: Zehr, E. I., ed. Methods for Evaluating Plant Fungicides, Nematicides, and Bactericides. Am. Phytopathol. Soc., St. Paul, MN. 141 pp.
3. CHAPMAN, R. W. 1960. The effects of *Meloidogyne incognita* and *M. hapla* on the growth of Kenland red clover and Atlantic alfalfa. Phytopathology 50:181-182.
4. CHI, C. C. 1966. Phytophthora root rot of alfalfa in Canada. Plant Dis. Rep. 50:451-453.
5. DAMIRGI, S. M., F. D. COOK, and G. P. WEBSTER. 1978. Incidence of root rot fungus in a diseased alfalfa soil in central Alberta. Can. J. Soil Sci. 58:229-236.
6. ELGIN, J. H., Jr., D. W. EVANS, and J. M. KRAFT. 1972. Occurrence of Phytophthora root rot of alfalfa in Washington. Plant Dis. Rep. 56:830-831.
7. ERWIN, D. C. 1954. Root rot of alfalfa caused by *Phytophthora cryptogea*. Phytopathology 44:700-704.
8. ERWIN, D. C. 1965. Reclassification of the causal agent of root rot of alfalfa from *Phytophthora cryptogea* to *P. megasperma*. Phytopathology 55:1139-1143.
9. FROSHEISER, F. I. 1969. Phytophthora root rot of alfalfa in the upper midwest. Plant Dis. Rep. 53:595-597.
10. FROSHEISER, F. I., and D. K. BARNES. 1973. Field and greenhouse selection for Phytophthora root rot resistance in alfalfa. Crop Sci. 13:735-738.
11. GRIFFIN, G. D. 1979. Root-knot nematodes. Pages 51-52 in: Graham, J. H., coordinator. A Compendium of Alfalfa Diseases. Am. Phytopathol. Soc., St. Paul, MN.
12. HUNT, O. J., G. D. GRIFFIN, J. J. MURRAY, M. W. PEDERSEN, and R. N. PEADEN. 1971. The effects of root-knot nematodes on bacterial wilt in alfalfa. Phytopathology 61:256-259.
13. IRWIN, J. A. G. 1974. Reaction of lucerne cultivars to *Phytophthora megasperma*, the cause of a root rot in Queensland. Aust. J. Exp. Agric. Anim. Husb. 14:561-565.
14. JOHNSON, H. W., and F. L. MORGAN. 1965. Phytophthora root and crown rot of alfalfa in the Yazoo-Mississippi Delta. Plant Dis. Rep. 49:753-755.
15. KUAN, T.-L., and D. C. ERWIN. 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybean and alfalfa. Phytopathology 70:333-338.
16. KUSHNER, V. D., and H. W. CRITTENDEN. 1967. Influence of potassium chloride on the interrelationship of fungi and a nematode in alfalfa roots. Phytopathology 57:646.
17. LEATH, K. T., and J. E. BAYLOR. 1975. Phytophthora root rot of alfalfa in Pennsylvania. Plant Dis. Rep. 59:972-974.
18. MATSUMOTO, N., and T. ARAKI. 1978. Alfalfa root rot caused by *Phytophthora megasperma* Drechsler in Japan. Ann. Phytopathol. Soc. Jpn. 44:214-217.
19. McGLOHON, N. E., J. N. SASSER, and R. T. SHERWOOD. 1971. Investigations of plant-parasitic nematodes associated with forage crops in North Carolina. N.C. Agric. Exp. Stn. Tech. Bull. 148. 39 pp.
20. MOORE, E. L., P. N. DROLSOM, F. A. TODD, and E. E. CLAYTON. 1956. Black shank resistance in flue-cured tobacco as influenced by tolerance to certain parasitic nematodes. Phytopathology 46:545-550.
21. POWELL, N. T. 1971. Interactions between nematodes and fungi in disease complexes. Annu. Rev. Phytopathol. 9:253-274.
22. POWELL, N. T., and C. J. NUSBAUM. 1960. The black shank-root-knot complex in flue-cured tobacco. Phytopathology 50:899-906.
23. STOVOLD, G. E., and D. E. CARRATT. 1978. Occurrence of *Phytophthora megasperma* in alfalfa growing soils of New South Wales. Plant Dis. Rep. 62:742-744.
24. TSAO, P. H., and M. J. GARBER. 1960. Methods of soil infestation, watering and assessing the degree of root infection for greenhouse in situ ecological studies with citrus phytophthoras. Plant Dis. Rep. 44:710-715.
25. WELTY, R. E., and T. H. BUSBICE. 1976. Phytophthora root rot of alfalfa in North Carolina. Plant Dis. Rep. 60:14-18.
26. WYLLIE, T. D., and D. P. TAYLOR. 1960. Phytophthora root rot of soybeans as affected by soil temperature and *Meloidogyne hapla*. Plant Dis. Rep. 44:543-545.